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Supporting Information

Postsynthetic Functionalization of DNA-Nanocomposites with Proteins Yields Bioinstructive Matrices for Cell Culture Applications

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Materials and Methods

Synthesis of multifunctional silica nanoparticles

To prepare silica nanoparticles (SiNPs), cyclohexane (38 mL, VWR), 1-hexanol (9 mL, VWR) and triton X-100 (9 mL, AppliChem) were mixed vigorously in a 250 mL round-bottom glass bottle. Double distilled water (2 mL) was added to the mixture to produce stable reverse micelles. After mixing for 10 min, Tetraethyl orthosilicate (TEOS, 500 μL, Sigma-Aldrich) was added to the mixture followed by the addition of ammonia solution (28-30%, 500 μL, VWR). This mixture was stirred at room temperature for 24 h. Subsequently, additional TEOS (250 μL) was added to the mixture, and after stirring for 30 min, 3-(trihydroxysilyl)propyl methylphosphonate (THPMP, monosodium salt solution, 200 μL, Sigma-Aldrich) and N1-(3-trimethoxysilylpropyl)diethylenetriamine (DETAPTMS, 50 μL, Sigma-Aldrich) were added to modify the surface of the nanoparticles with negatively charged phosphonate and amino groups. The mixture was allowed to react for 24 h and subsequently (3-mercaptopropyl) trimethoxysilane (MPTMS, 30 μL, Alfa Aesar) was added to modify the nanoparticle's surface with thiol groups. The mixture was stirred at room temperature for an additional 3 h. The micelles were broken with acetone, and the resultant nanoparticles were centrifuged and washed at least 5 times with absolute ethanol, and finally dispersed in PBS buffer (23 mM KH_2PO_4, 77 mM K_2HPO_4, 50 mM NaCl, pH 7.4) to a final concentration of 10 mg/mL.

Immobilization of PEG and ssDNA on silica nanoparticles

To install PEG groups on the surface of SiNP, tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 0.5 M solution, 8.0 μL, Sigma-Aldrich) was added to 1 mL PBS solution of SiNP (10 mg/mL) to reduce any disulfide bonds. Subsequently, a DMSO solution of mPEG-maleimide (50 mg/mL, 10 mL, molecular weight of ~2000, Sigma-Aldrich) was added to the mixture. After incubation at room temperature overnight, the modified nanoparticles were purified by centrifugation and re-dispersion with PBS buffer for 3-5 times.

Next, amino-modified primer (aP, Table S1, all oligonucleotides used in this research were from Sigma-Aldrich.) was covalently immobilized on the particle surface *via* glutaraldehyde coupling. Typically, PEGylated SiNP (10 mg/mL, 1.0 mL) in PBS buffer were mixed with glutaraldehyde (50 % in water, 250 μL, VWR), and the mixture was stirred at room temperature for 1 h. The resultant nanoparticles were washed 3 times with PBS buffer, re-dispersed in PBS buffer (1.0 mL) and mixed with aP (100 μM, 50 μL). The mixture was incubated at room temperature for 12 h. Subsequently, glycine (0.4 M, 1.0 mL, AppliChem) was added to block any unreacted aldehyde groups, followed by addition of sodium cyanoborohydride (60 mM, 400 μL, Sigma-Aldrich) to reduce Schiff's bases into stable secondary amines. The primer-modified SiNPs are denoted as SiNP-P.

Synthesis of SiNP-DNA nanocomposite hydrogel

The linear ssDNA (T, Table S1) phosphorylated at the 5' end was circularized through hybridization with P attached on the surface of SiNP-P using T4 DNA ligase. To this end, linear ssDNA (T, 10 μM, 30 μL) and 10× T4 DNA ligation buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM ATP, 100 mM dithiothreitol (DTT), 7.5 μL) were added to 60 μL SiNP-P suspension (10 mg/mL), and the mixture was incubated at 25 °C for 3 h. After addition of 2.5 μL T4 DNA ligase

(400,000 U/mL, New England Biolabs), the mixture was further incubated at 25 °C for more than 3 h to ligate the nicked ends of the template, leading to the formation of particle-primer-template (SiNP-P-T) complexes. The RCA reaction mixture contained dNTPs (10 mM, 10 µL, New England Biolabs), 10× BSA (10 mg/mL, 5 µL), 10× phi29 DNA polymerase buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 40 mM DTT, pH 7.5, 5 µL) and phi29 DNA polymerase (10,000 U/mL, 5 µL, New England Biolabs). The polymerisation was initiated *via* the addition of 50 µL of the purified SiNP-P-T. After incubation at 30 °C for 48 h, the formed SiNP-DNA nanocomposite hydrogels were purified by carefully replacing the reaction buffer with PBS for 5-7 times and stored at 4 °C before use. With a final SiNP-P concentration of 4 mg/mL, the formed SiNP-DNA nanocomposite hydrogel was denoted as S^{BL} nanocomposite material from which 5'-$(ATG)_4$-3' sequence in the loop (L, Table S1) of DNA backbone is complementary with the DNA sequence cL (5'-$(CAT)_8$-3') in the protein-DNA conjugate or the binding tag of DNA origami nanostructure (see below).

As a control, SiNP-P1 were synthesized, hybridized with T1, and polymerized via RCA as described above (for the sequences of P1 and T1, see Table S1). The formed hydrogel was used as a negative control material, denoted as S^{NC}, where the DNA sequence in the loop (L1, Table S1) of DNA backbone is not complementary with the DNA sequence (cL, Table S1) in the protein-DNA conjugates or the binding tags of the DNA origami nanostructures (see below).

Synthesis of SiNP/CNT-DNA nanocomposite hydrogel

428 µL aqueous dispersion containing 1.2 mg single-walled carbon nanotubes (CNT, 1 µm length, 0.83 nm diameter, Sigma-Aldrich) were mixed with 344 µL aqueous solution of ssDNA oligonucleotide (P, 100 µM, Table S1) and 428 µL aqueous solution of NaCl (0.28 µM), followed by ultrasonication on ice for 90 min at a power of approx. 10 W using a Ultrasonic Cleaner (VWR). The resulting products were centrifuged at 16,000 × g and 4 °C for 90 min to remove CNT aggregates. The free DNA was removed by ultrafiltration at 4000 × g and 4 °C for 10 min using an ultrafiltration unit Vivaspin 6 with a molecular weight cut-off (MWCO) of 50 kD (Sartorius Stedim Biotech), and the primer modified CNT (CNT-P) were re-dispersed from the filtration membrane using distilled water. The purification process was repeated several times until no free DNA could be detected in the flow through.

Mixtures containing SiNP-P (4 mg/mL) and CNT-P (160 µg/mL) were subjected to ligation with RCA template (T) and subsequent RCA polymerization, similar as described above. After incubation for 48 h, the formed SiNP/CNT-DNA composite hydrogel bearing repetitive binding loops (SC^{BL}) was purified by carefully replacing the reaction buffer with PBS for 5-7 times and stored at 4 °C before use.

Preparation of protein-DNA conjugates

The synthesis and purification of the covalent streptavidin (STV)-DNA or vectibix (VEC)-DNA conjugate was carried out using thiolated oligonucleotides (tcL, Table S1) and STV or VEC, as previously described.[1] In brief, VEC (20 nmol, Amgen) or STV (20 nmol) was derivatized with maleimide groups using the heterobifunctional crosslinker sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sSMCC, 30 equivalents to VEC or 300 equivalent to STV, Thermo Fisher Scientific). The activated proteins were then allowed to react with the reduced thiolated oligonucleotide (10 nmol) and subsequently purified by anion exchange

chromatography. The one-to-one molar ratio of oligonucleotide and protein moiety of the conjugate was verified by fast protein liquid chromatography (FPLC, see below) and polyacrylamide gel electrophoresis (PAGE, see below). Conjugate concentration was determined by absorbance measurements.[2]

Chromatographic characterization and purification of protein-DNA conjugates

To purify the protein-DNA conjugates, an anionic exchange column (MonoQ5/50 GL column, GE Healthcare Life Science) was coupled to an automated FPLC-system (Äkta pure, GE Healtcare). The fractions were collected and stored at 4° C before use.

Polyacrylamide gel electrophoresis

Protein-DNA conjugates (7 pmol, 12.5 µL) were loaded into a native polyacrylamide gel (8.5%) or gradient polyacrylamide gel (4-15% Mini-PROTEAN® TGX™, Bio-Rad) (Tris-glycine buffer, 25 mM Tris, 192 mM glycine, pH 8.3), run with a current of 40 mA for 70 min, imaged under Cy3 channel for visualization of DNA, and stained with Coomassie for protein visualization.

Design of the DNA origami nanostructure

The rectangular DNA origami nanostructure (DON) used in this work was assembled from the single stranded scaffold p7560 (Tilibit nanosystems) and 240 staple strand oligonucleotides. The general design was adapted from Rothemund's design.[3] It was twist-corrected by deleting one base from every fourth column of staples and modified to realize the selective installment of fluorophore-labeled or biotinylated staples for STV binding on the upper side and single-stranded binding tags on the bottom side of the quasi-2D DON rectangle. With "lower" and "upper side", we refer to the position on DON plane after binding on a surface. The sequences of unmodified staple strands are listed in Table S3. Several positions were chosen on the DON's upper side for the installment of Cy3 (20) or biotin groups (5) and nine positions on the lower side were selected for the tethering of single-stranded binding sites. These variations were realized by exchange of respectively modified staples, as specified in Table S4.

Assembly and purification of biotinylated DNA origami nanostructures

The DONs were assembled according to Rothemund's procedure, using a 1:10 molar ratio between the scaffold strand p7560 and each of the staple strands. The assembly was conducted in TE-Mg buffer (20 mM Tris base, 1 mM EDTA, 6 mM $MgCl_2$, pH 7.6) in a total volume of 500 µL on a Thermocycler (Eppendorf Master cycler® pro) by a step-wise temperature decrease from 75°C to 25°C. After an initial denaturing step at 95 °C for 5 min, the temperature was decreased at -1 °C/step for 50 steps and each step was held for 10 s. After annealing, excess staple strands were removed by PEG precipitation according to Dietz's procedure.[4] DONs were precipitated by adding a 1:1 volume ratio of precipitating buffer (5 mM Tris base, 1 mM EDTA, 505 mM NaCl, 15% PEG-8000), followed by centrifugation at 16000 g for 30 min. The obtained pellet was re-suspended in 50 µL TE-Mg buffer (20 mM Tris base, 1 mM EDTA, 6 mM $MgCl_2$, pH 7.6).

Quantification of DNA origami nanostructures *via* qPCR

The concentration of the purified DONs was determined by quantitative PCR (qPCR). A calibration curve was generated from serial 1:10 dilutions of the p7560 plasmid in the range of 15 nM-150 fM. 10 mL of PCR mixture were prepared by mixing 1 mL 10× PCR buffer (160 mM ammonium sulfate, 670 mM Tris-HCl, 0.1% Tween 20, pH 8.8), 500 µL KCl (50 mM), 600 µL $MgCl_2$ (50 mM), 200 µL dNTPs (10 mM each), 100 µL primer FW_p7560 (100 µM, CCAACGTGACCTATCCCATTAC), 100 µL primer RV_p7560 (100 µM, TTCCTGTAGCCAGCTTTCATC), 20 µL TaqMan3_p7560 probe (100 µM, FAM-CGACGGGTTGTTACTCGCTCACAT-TAMRA) and 100 µL Taq DNA Polymerase (5 U/µL) (New England Biolabs) in autoclaved H_2O. 20 µL of the PCR mixture were pipetted in each well of a PCR microplate and 1.5 µL of p7560 calibration standards or DON samples were added. qPCR was performed using a real-time thermocycler (Corbett research). The threshold cycle (Ct) was manually adjusted. ΔCt values were calculated by subtraction of the Ct signal from the maximal number of cycles (C_{Max}). To calculate the concentration of the DON samples, the ΔCt values were plotted against the log concentration of the p7560 calibration samples and a linear regression was used for quantification.

AFM analysis

The samples were diluted in TE-Mg (20 mM Tris base, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 7.6) up to 20 times, depending on the sample concentration. 10 µL of diluted samples were deposited on freshly cleaved mica surface (Plano GmbH) and allowed to adsorb at room temperature for 3 min. After addition of 50 µL 1× TAE-Mg (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM Mg acetate, pH 8.0), the samples were imaged with pyramidal tips (SNL-10 tips, radius 2 nm, spring constant 0.35 N/m, Bruker) using a NanoWizard 3 atomic force microscope (JPK) under a force-curve based imaging mode (QI^{TM}). The obtained images were analyzed by using the JPK data processing software.

Postsynthetic modification of DNA nanocomposite hydrogel using protein-DNA conjugates or protein-DNA origami nanostructures (DONs)

The purified S^{BL} or SC^{BL} materials were immersed in TE-NaCl buffer (20 mM Tris base, 1 mM EDTA, 750 mM NaCl, pH 7.6). For hybridization of VEC-cL^{Cy3} conjugate, a stock solution of VEC-cL^{Cy3} conjugate was gently pipetted into the 96 well plate (cover glass bottom, MoBiTec) or ibidi petri dish (polymer coverslip, 4 well silicone insert, Ibidi) containing S^{BL} or SC^{BL} materials to a final concentration of 10 nM, and then incubated at room temperature for 24 h. The resultant S^{BL} or SC^{BL} materials bearing VEC were washed with PBS for 5-7 times and stored at 4° C before use.

For hybridization of epidermal growth factor (EGF)-STV-cL^{Cy3} or EGF-STV-DON^{Cy3}, S^{BL} or SC^{BL} materials were incubated with the EGF-STV-cL^{Cy3} conjugate (final concentration of STV-cL^{Cy3}: 10 nM) or the EGF-STV-DON^{Cy3} (final concentration of DON: 2 nM) under the same

conditions as described above. Prior to the hybridization step, EGF-STV-cL^{Cy3} conjugate or EGF-STV-DON^{Cy3} construct was prepared according to the previous report.[5] Specifically, STV-DON^{Cy3} was prepared by mixing 5 μL of STV (10 μM in PBS) and 3 μL of biotinylated DON^{Cy3} solution (100 nM in TE-NaCl) at room temperature for 1 h. Then biotinylated epidermal growth factor (bEGF, 90 pmol in 10 μL PBS buffer) was incubated with the raw product of STV-DON^{Cy3} for another 1 h to obtain EGF-STV-DON^{Cy3}. Likewise, bEGF (2.7 pmol in 10 μL PBS buffer) was incubated with STV-cL^{Cy3} (100 nM in TE-NaCl, 15 μL) at room temperature for 1 h to obtain EGF-STV-cL^{Cy3}.

Immunofluorescence staining of protein-DNA conjugate or protein-DNA origami nanostructure (DON) construct modified SiNP-DNA nanocomposite hydrogel

To verify the successful postsynthetic modification, the VEC-cL^{Cy3} conjugate, EGF-STV-cL^{Cy3} conjugate, or EGF-STV-DON^{Cy3} modified S^{BL} materials were subjected to immunofluorescence staining. Briefly, S^{BL} materials bearing VEC-cL^{Cy3} were incubated with a fluorophore conjugated antibody (FITC conjugated pAb Rabbit anti-Human IgG (H+L) Secondary Antibody Thermo Fisher Scientific SA1-36099, diluted 1:40) in BSA (1 mg/mL, in PBS) at room temperature for 4 h. The materials were washed with PBS to remove the excess fluorophore conjugated secondary antibody and then visualized by fluorescence microscopy (Axiovert 200M, Carl Zeiss).

For the immunofluorescence staining of EGF, S^{BL} materials bearing EGF-STV-cL^{Cy3} or EGF-STV-DON^{Cy3} were incubated with primary antibody (pAb rabbit anti-EGF, Abcam ab9695, diluted 1:300) in BSA (1 mg/mL, in PBS) at 4 °C overnight. Subsequently, the materials were washed several times with PBS to remove free primary antibody and incubated with fluorophore conjugated secondary antibody (Alexa Fluor® 647 conjugated Goat anti-Rabbit IgG Secondary Antibody, Life technology A21244, diluted 1:300) in BSA (1 mg/mL, in PBS) at room temperature for 4 h. The materials were washed with PBS to remove the excess fluorophore conjugated secondary antibody and then visualized by fluorescence microscopy (Axiovert 200M, Carl Zeiss).

Quantification of protein-DNA conjugates or DNA origami nanostructures (DONs) in the SiNP-DNA nanocomposite hydrogel

The modification of STV-cL and VEC-cL in the S^{BL} materials was further quantified *via* western blotting. After 24 h incubation of conjugates in S^{BL} materials (see "Postsynthetic modification of SiNP-DNA nanocomposite hydrogel using protein-DNA conjugates"), the supernatants were collected to determine the remaining concentration of the conjugates. To initially generate a calibration curve, stock solutions of STV-cL or VEC-cL conjugates of various concentrations (0.65-20 nM in 10 μL Tris-glycine buffer) were prepared. All solution samples were loaded into a polyacrylamide gel (see "Polyacrylamide gel electrophoresis"). Following, the STV or VEC moieties in the polyacrylamide gel were detected *via* western blotting using alkaline phosphatase (AP)-conjugated biotin (Rockland Immunochemicals, diluted 1:1000) or AP-conjugated Rabbit anti-Human IgG H&L (Abcam ab6760, diluted 1:2000), respectively, and developed with the AP conjugate substrate kit (Bio-Rad). The concentration of unbound conjugates in the supernatant was calculated from the calibration curve of band intensity extracted by the software ImageJ.

To quantify the DON in the nanocomposite materials, the concentration of unbound DON in the supernatant was determined by qPCR (see "Quantification of DNA origami nanostructures *via* qPCR").

Routine cell culture

MCF7 breast cancer cells stably transfected to overexpress the EGF receptor (EGFR) have been used before in our lab to study the activation of the EGFR by EGF presented on solid 2D surfaces.[5] Our previous studies had shown that these cells are well characterized, they enable direct microscopy observation of the cellular membrane and the overexpression of the enhanced green fluorescent protein (eGFP)-tagged EGFR has no negative effect on the cell behavior. Based on this experience, we found it straightforward to investigate the potential activation/inhibition of EGFR in the protein-modified 3D matrix using the same cell line.

Human $MCF7_{eGFP}$ breast cancer cells stably transfected to express the EGFR fused to the eGFP (eGFP-EGFR) were obtained from the Max-Planck Institute for Molecular Physiology (Dortmund). The cells were cultured in 25 cm^2 tissue culture flask (Corning) with $MCF7_{eGFP}$ medium, comprised of Eagle's minimum essential medium (EMEM, Gibco Laboratories), 1% penicillin/streptomycin (Life Technology), 10% FBS (Biochrom) and 0.6% G418 disulfate salt solution (50 mg/mL in water, Sigma-Aldrich) at 37 °C in a 5% CO_2 environment. The cells were washed twice with PBS (-/-) (without calcium and magnesium) and trypsinated by addition of 500 µL 0.25% Trypsin solution in PBS-EDTA (PBS with 0.02% EDTA, Biochrom) for 3 min. The trypsin activity was blocked by addition of 4.5 mL of fresh $MCF7_{eGFP}$ medium. The cells were passaged every 2 to 3 days.

Seeding cells in DNA nanocomposite hydrogels bearing EGF or VEC

12,000 cells in 200 µL medium were seeded in DNA nanocomposite hydrogels bearing EGF or VEC and allowed to grow in the materials at 37 °C for 2 h. For immunofluorescence staining of activated EGFR, the cell-loaden materials were washed with PBS for 3-5 times and fixed with 4% PFA (Polysciences) in PBS for 30 min. After permeabilization with a triton X-100 solution (0.1%, in PBS) for 1 h, 100 µL BSA solution (1 mg/mL, in PBS) of a primary antibody αP-EGFR (mAb rabbit anti-phospho Y1068 EGFR, Abcam ab32430, diluted 1:300) were added and incubated at 4 °C overnight. Subsequently, the materials were washed several times with PBS to remove the free primary antibody and then incubated with fluorophore conjugated secondary antibody (Alexa Fluor® 647 conjugated Goat anti-Rabbit IgG Secondary Antibody, Life technology A21244, diluted 1:300) in BSA (1 mg/mL, in PBS) at room temperature for 4 h. Eventually, the cells in the materials were counterstained with DAPI (1:1000) in PBS and washed several times with PBS for fluorescence microscopy inspection (LSM 880, Carl Zeiss).

Supplementary Figures

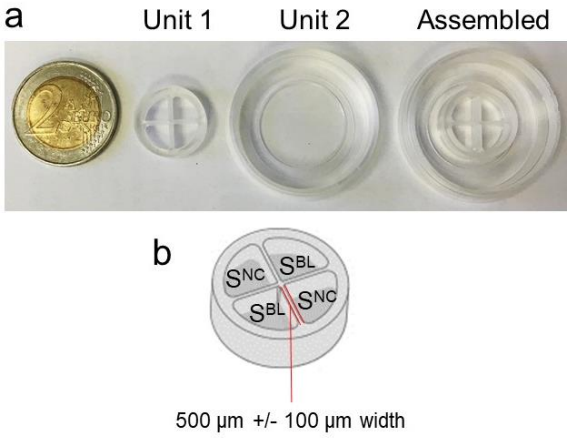

Figure S1. (a) Photographs of the commercial 4-well silicone gasket (unit 1), the coverslip bottomed petri dish (unit 2) and their glued assembly. For size comparison, a 2-Euro coin is shown in the left. (b) Schematics of the experimental setup, shown in Figure 1, main text. Individual wells of the 4-well silicone gasket containing S^{BL} and S^{NC} materials are separated by the silicone barrier of 500 µm +/-100 µm thickness between two wells.

Discussion

To clarify the differences in the **mechanical properties between the SiNP-DNA composite materials and other DNA hydrogels**, some comments are provided in the following. DNA hydrogels are usually prepared by two methods, either the hybridization/ligation of linear and branched DNA oligonucleotide building blocks, or else by enzymatic extension of oligonucleotide primers, in particular through rolling circle amplification (RCA).[6] In the present work, RCA method was used to prepare the SiNP-DNA nanocomposite materials whose mechanical stiffness is similar to that of previously reported pure DNA hydrogels produced by RCA, with a shear modulus value ranging from several to tens of Pa.[7] Through the integration of carbon nanotubes (CNTs), which are homogeneously incorporated into the composite material during the RCA process, the mechanical properties can be systematically changed, as we have previously investigated using rheological methods.[8] In summary, the incorporation of CNTs leads to an increased mechanical stiffness of the composites. However, the shear modulus values of the RCA-based SiNP-DNA and SiNP/CNT-DNA materials are substantially lower than that of DNA hydrogels produced by the hybridization/ligation method (shear modulus values range from hundreds to thousands of Pa).[9]

To clarify the differences in the **synthetic complexity and costs between DNA hydrogel and traditional polymer hydrogel**, some comments are provided in the following. In terms of synthetic complexity, the SiNP-DNA nanocomposites described here were synthesized by the RCA method, which is a rather versatile, controlled and straightforward methodology for

polymerization under mild biochemical conditions. In contrast, the synthesis of traditional polymer hydrogels generally occurs under comparably harsh chemical conditions and usually requires an excess of chemicals that has be removed afterwards by multiple purification steps, leading otherwise to potential cytotoxicity. In terms of costs, DNA molecules are more expensive than traditional polymers. Hence, DNA-based hydrogels generally are more expensive than traditional polymer hydrogels. However, it needs to be taken into account that the RCA method allows to enzymatically synthesize large amounts of DNA from relatively cheap dNTP precursors, thereby enabling the production of DNA-based hydrogels with lower costs than the polymerase-free methods for preparation of DNA-based hydrogels.

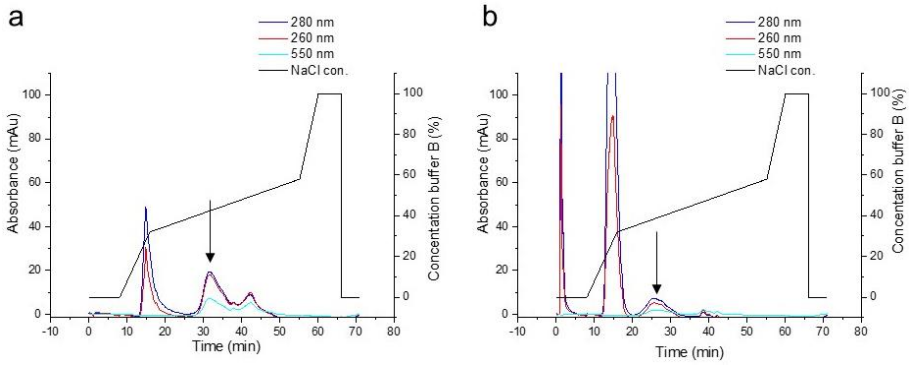

Figure S2. FPLC chromatograms of the covalent protein-DNA conjugates STV-cL^{Cy3} and VEC-cL^{Cy3}. The arrows point at the one-to-one molar ratio conjugate consisting of the oligonucleotide and the protein: (a) STV-cL^{Cy3} conjugate and (b) VEC-cL^{Cy3} conjugate, respectively, which were collected for further use. Absorbance: STV or VEC, 280 nm; DNA, 260 nm; Cy3, 550 nm.

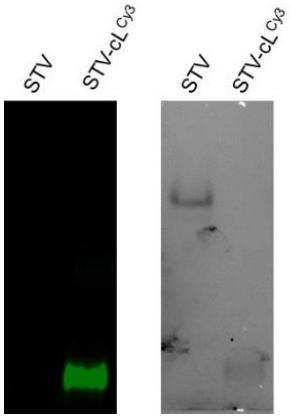

Figure S3. Gel-electrophoretic characterization of the covalent STV-DNA conjugate in a native polyacrylamide gel (8.5%). After electrophoresis, the gel was imaged based on the Cy3 fluorescence for DNA visualization (left panel) and then stained with Coomassie for protein visualization (right panel). Note that the formation of STV-cL^{Cy3} conjugate is indicated by the shifted band, as compared to native STV.

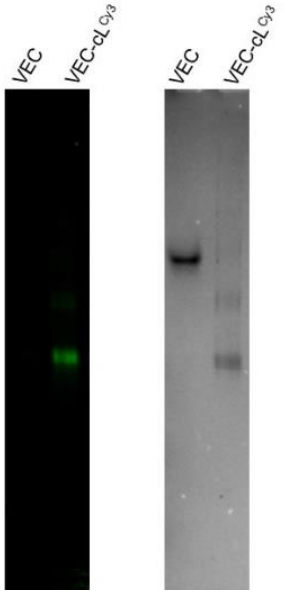

Figure S4. Gel-electrophoretic characterization of covalent VEC-DNA conjugate in a gradient polyacrylamide gel (4-15%). After electrophoresis, the gel was imaged based on the Cy3 fluorescence for DNA visualization (left panel) and then stained with Coomassie for protein visualization (right panel). Note that the formation of VEC-cL^{Cy3} conjugate is indicated by the shifted band, as compared to native VEC.

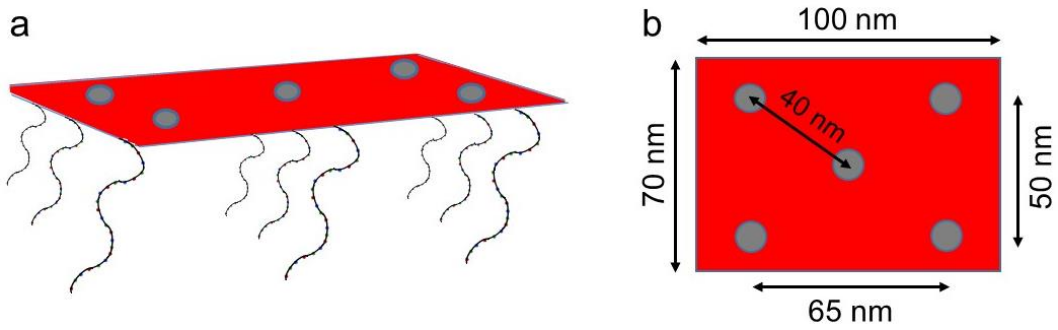

Figure S5. Schematic illustration of the fluorescent DNA origami nanostructure (DON^{Cy3}) bearing 5 biotin groups and nine single-stranded protruding arms for selective binding of STV and S^{BL} materials, respectively. (a) Perspective view and (b) top view with indication of the geometrical characteristics for the arrangement of the biotin groups.

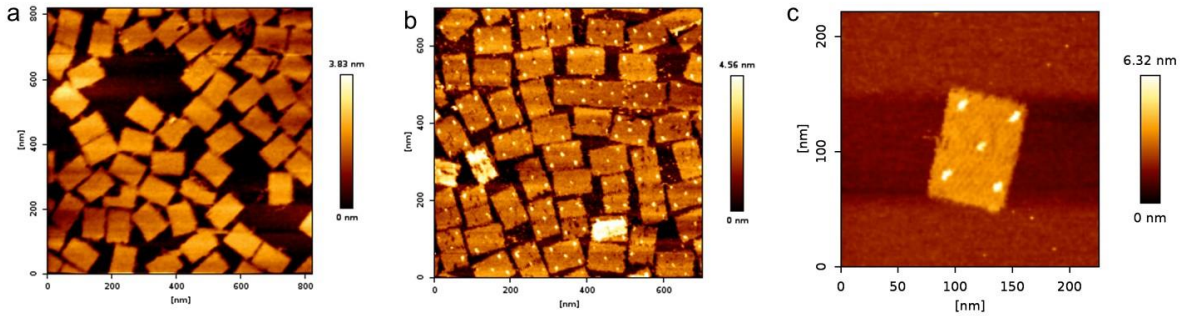

Figure S6. AFM analysis of STV-DON. Representative AFM images of biotinylated DON (a) before and (b, c) after STV functionalization. STV surface occupancy (%) was statistically calculated to be 80.5%.

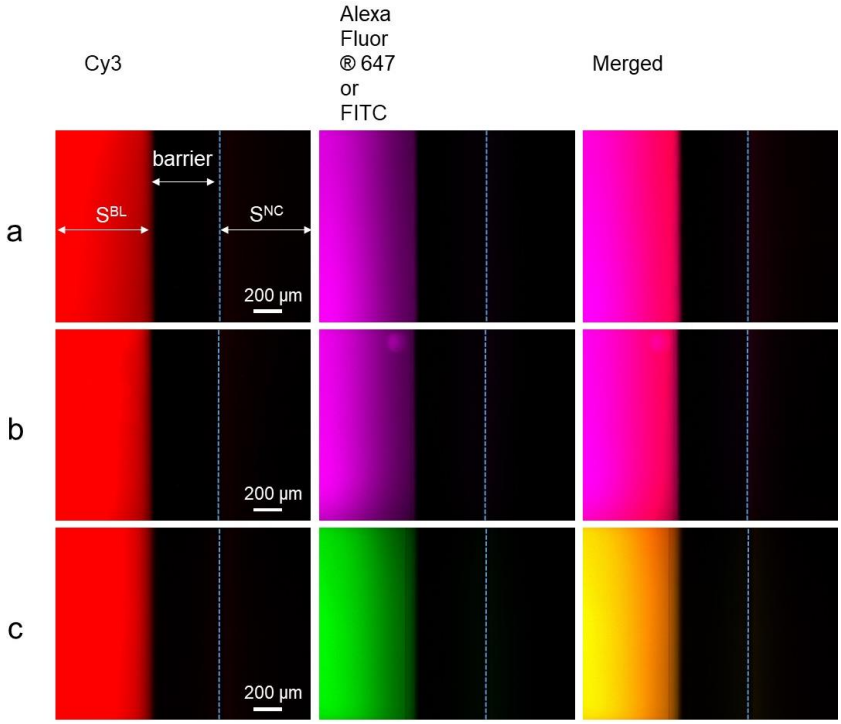

Figure S7. Steric accessibility of the EGF and VEC moieties inside the S materials, as determined by immunostaining with specific antibodies. Shown are representative fluorescence WF microscopy images of S^{BL} and S^{NC} materials modified with EGF-STV-cL^{Cy3} (a), EGF-STV-DON^{Cy3} (b) and VEC-cL^{Cy3} (c) after staining with polyclonal rabbit anti-EGF primary antibody and Alexa Fluor® 647-conjugated goat anti-rabbit IgG secondary antibody (a, b) or FITC-conjugated polyclonal rabbit anti-human IgG (H+L) antibody (c). Red: Cy3, magenta: Alexa Fluor® 647, green: FITC. Note that neither hybridization of protein-DNA conjugates nor binding of detection antibodies occurred in the S^{NC} materials.

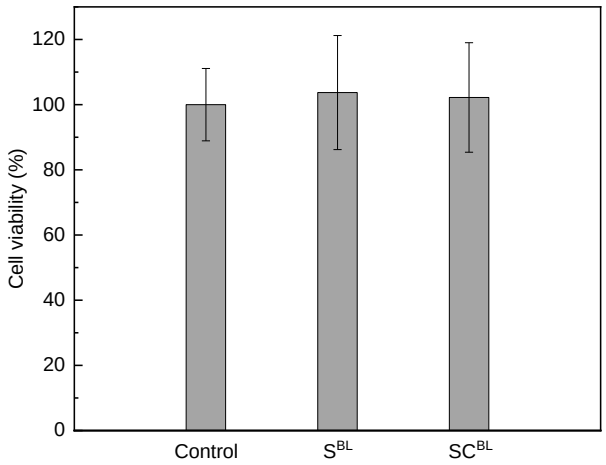

Figure S8. Cytocompatibility of DNA nanocomposite materials. CCK-8 viability assay after incubation of MCF7 cells for 24 h with the S^{BL} and SC^{BL} materials or, for control, with PBS buffer only.

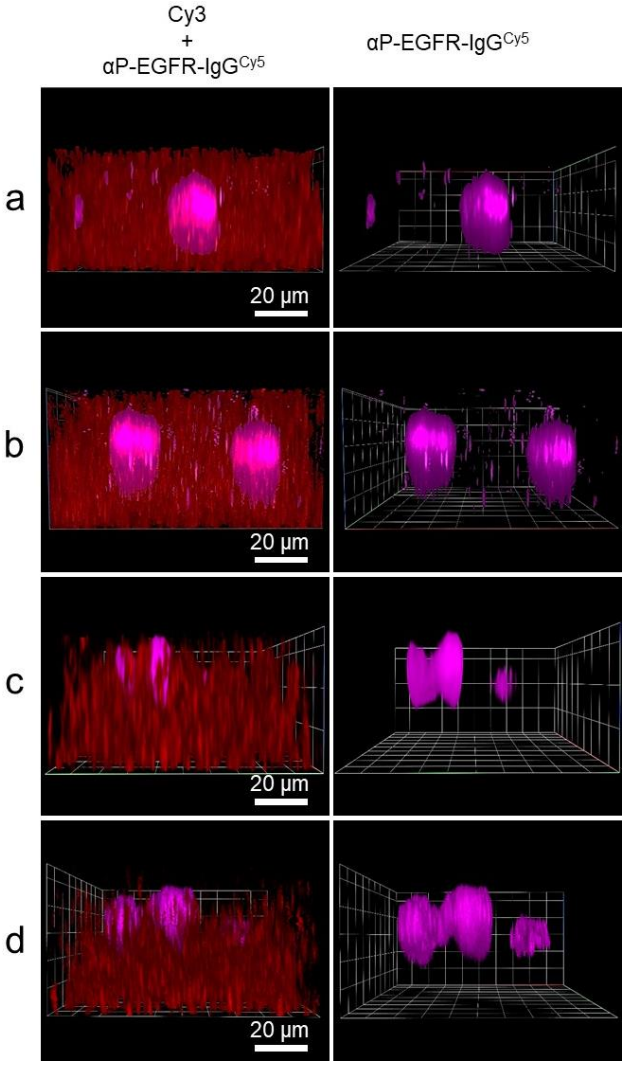

Figure S9. Z-stack 3D CLSM analysis of EGFR phosphorylation of MCF7$_{eGFP}$ cells grown in (a, b) S^{BL} or (c, d) SC^{BL} materials that were modified with (a, c) EGF-STV-cL^{Cy3} or (b, d) EGF-STV-DON^{Cy3}. Note that phosphorylation of EGFR (cyan) depends on the ligands incorporated via postsynthetic modification.

Discussion

As observed in our previous work,[8] MCF7 cells can transmigrate through the soft matrix of SiNP-DNA nanocomposites, and we find a similar behavior for the S^{BL} materials (a, b), as expected. In contrast, we found in our previous work,[8] that cells cannot transmigrate through DNA nanocomposites, when these are reinforced with CNT, i.e., when the mass ratio of SiNP:CNT $\leq$ 25, as it is the case for the SC^{BL} materials (c, d). Owing to the increased entanglement and mechanical stiffness of these SC materials as compared to S materials (G_0 of 8.5 Pa or 3.2 Pa, respectively),[8] the cells rather dig themselves into the upper layer of the SC materials. Therefore, we selected SiNP-DNA (S^{BL}) and SiNP/CNT-DNA (SC^{BL}, mass ratio of SiNP:CNT = 25:1) as representative materials to demonstrate that nanocomposites with different composition, mechanical stiffness and viscosity can be functionalized with proteins to enable cell culture applications.

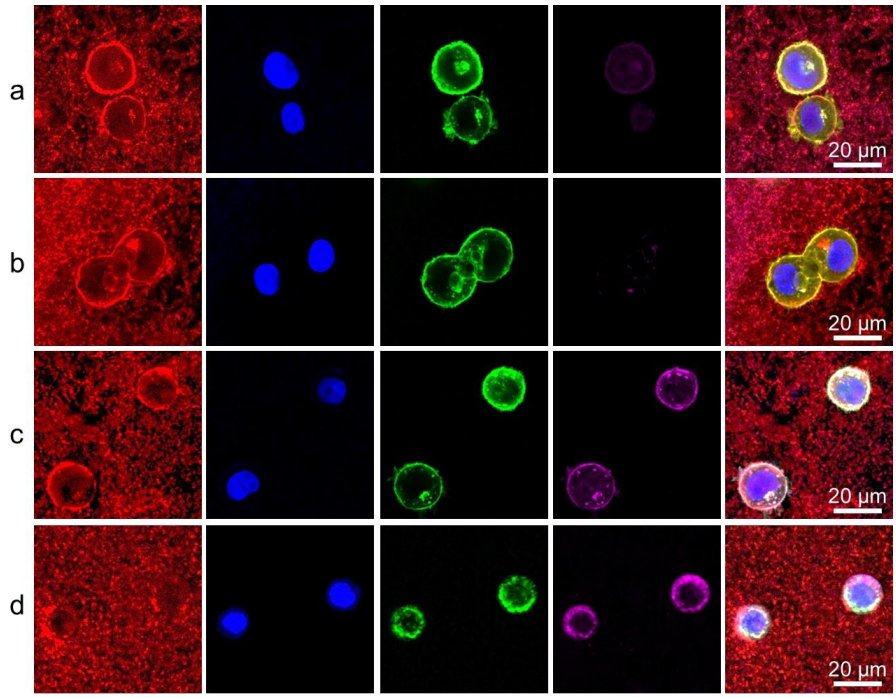

Figure S10. CLSM analysis of EGFR phosphorylation of $MCF7_{eGFP}$ cells grown in the (a) cL^{Cy3}, (b) VEC-cL^{Cy3}, (c) EGF-STV-cL^{Cy3}, or (d) EGF-STV-DON^{Cy3} modified CNT-reinforced SC^{BL} materials, respectively. For representative 3D images, see Figure S9.

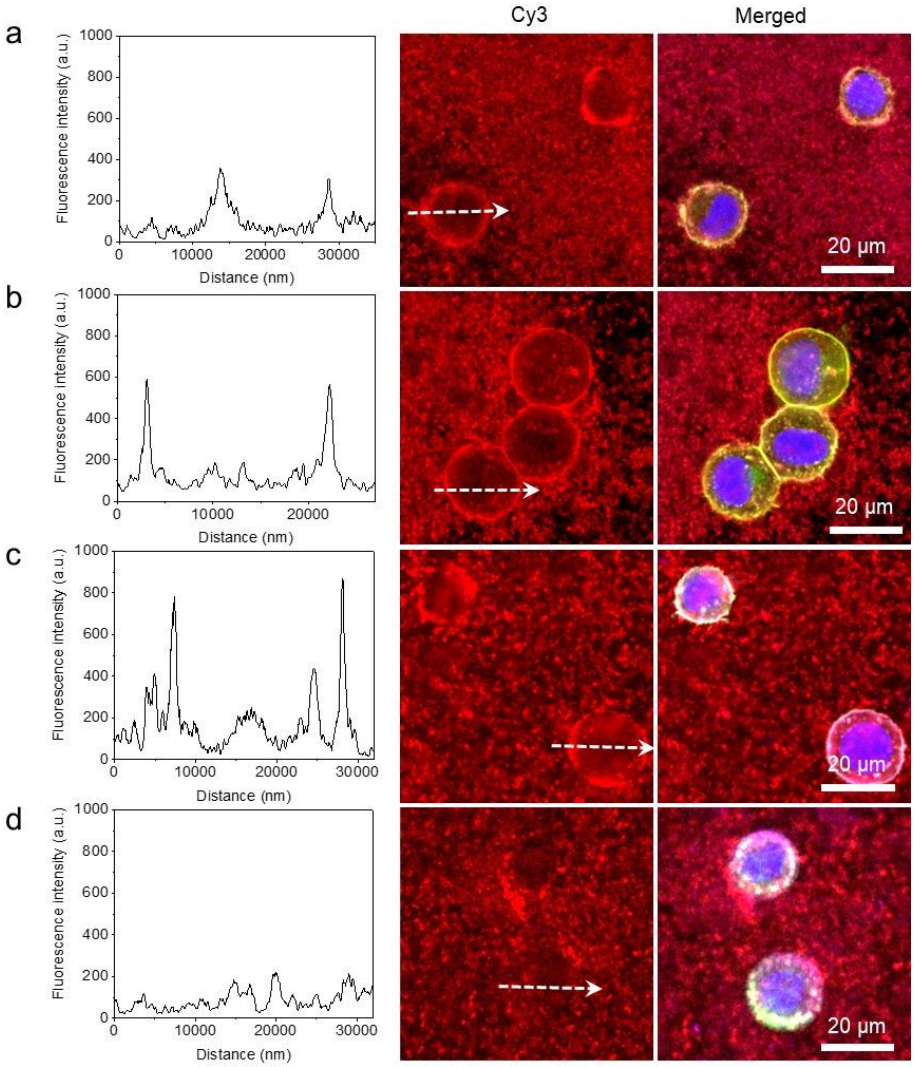

Figure S11. Fluorescence intensity profile analysis of the accumulation of ligand-modified S^{BL} materials by $MCF7_{eGFP}$ cells. Fluorescence intensity profiles of the dashed arrow lines obtained from CLSM images of $MCF7_{eGFP}$ cells grown in (a) cL^{Cy3}, (b) VEC-cL^{Cy3}, (c) EGF-STV-cL^{Cy3}, and (d) EGF-STV-DON^{Cy3} modified S^{BL} materials (Cy3 channel), respectively. Note that S^{BL} material is substantially less accumulated by cells in the case of EGF-STV-DON^{Cy3}- as compared to the cL^{Cy3}-, VEC-cL^{Cy3}-, or EGF-STV-cL^{Cy3}-modified materials. Also note that the same effect was observed for the analogous SC^{BL} materials (Figure S12).

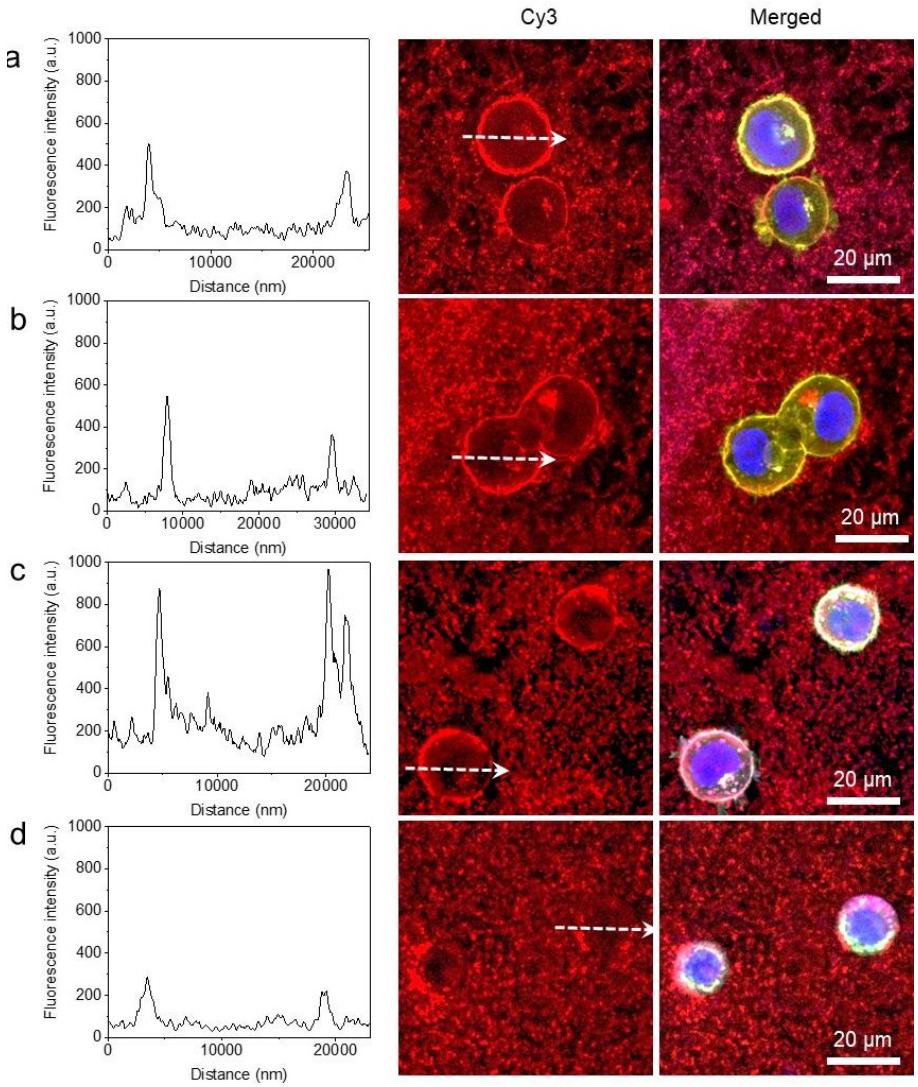

Figure S12. Fluorescence intensity profile analysis of the accumulation of ligand-modified SC^{BL} materials by $MCF7_{eGFP}$ cells. Fluorescence intensity profiles of the dashed arrow lines obtained from CLSM images of $MCF7_{eGFP}$ cells grown in (a) cL^{Cy3}, (b) VEC-cL^{Cy3}, (c) EGF-STV-cL^{Cy3}, and (d) EGF-STV-DON^{Cy3} modified SC^{BL} materials (Cy3 channel), respectively. Note that SC^{BL} material is substantially less accumulated by cells in the case of EGF-STV-DON^{Cy3}- as compared to the cL^{Cy3}-, VEC-cL^{Cy3}-, or EGF-STV-cL^{Cy3}-modified materials. Also note that the same effect was observed for the analogous S^{BL} materials (Figure S11).

Supplementary Tables

Table S1. List of sequences used for synthesis of DNA nanocomposite materials and protein-DNA conjugates.

Name[1-7)]	Sequence (5'-3')	Modification
aP	TTTTTTTTTTTTTCTAACTGCTGATGATGATGATGAAATA CTGTACGGTTAGA	5'Amine C12
P	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TCTAACTGCTGATGATGATGATGAAATACTGTACGGTTAGA	-
T	CATCATCATCATCAGCAGTTAGATGCTGCTGCAGCGATAC GCGTATCGCTATGGCATATCGTACGATATGCCGCAGCAGC ATCTAACCGTACAGTATT	5'Phosphorylation
aP1	TTTTTTTTTTTTTCTAACTGCTGCGCCGCCGGGAAAATAC TGTACGGTTAGA	5'Amine C12
T1	TTCCCGGCGGCGCAGCAGTTAGATGCTGCTGCAGCGATAC GCGTATCGCTATGGGTAACCGTACGGTTACCCGCAGCAGC ATCTAACCGTACAGTATT	5'Phosphorylation
L	CTGATGATGATGATGAATACTGTA	-
L1	CTGCGCCGCCGGGAAAATACTGTA	-
cL	CATCATCATCATCATCATCATCAT	3'Cyanine3
tcL	CATCATCATCATCATCATCATCAT	5'Thiol-Modifier C6 S-S; 3'Cyanine3

1) aP and T represent the primer and template, respectively, used for synthesis of S^{BL} materials;
2) aP/P and T represent the primer and template, respectively, used for synthesis of SC^{BL} materials;
3) aP1 and T1 represent the primer and template, respectively, used for synthesis of S^{NC} materials;
4) L represents the loop sequence in the DNA backbone of S^{BL} materials;
5) L1 represents the loop sequence in the DNA backbone of S^{NC} materials;
6) cL represents the complementary sequence to L;
7) tcL represents thiolated cL.

Table S2. Modification levels of S^{BL} materials obtained for hybridization with complementary DNA oligonucleotide, STV-DNA, VEC-DNA, and DON.

Materials	L	cL	STV-cL	VEC-cL	DON
nM	~5800 ± 1900[a)]	35 ± 10[b)]	20 ± 3[c)]	17 ± 3[c)]	5 ± 2[d)]
molecules/μm^3	~3500 ± 1100[a)]	21 ± 6[b)]	12 ± 2[c)]	10 ± 2[c)]	3 ± 1[d)]

a) L denotes the total number of loops in the polymerized S^{BL} materials. For estimation of the amount of loops, the amount of total dNTPs in the nanocomposites was determined by digestion of DNA strands using DNase I at the final concentration of 20 U/mL at 37 °C for 12 h. The amount of total dNTPs was then calculated by using a calibration curve (concentration *vs* absorbance) of dNTPs after subtracting the absorbance of DNase I, DNase I buffer, and dNTPs of primers on SiNPs from the overall absorbance. The total amount of DNA was then divided by the length of the loop (24 nt) to calculate the number of repeat units and thus the binding sites for the conjugates. From this, the concentration was calculated taking into account the volume of the S^{BL} material.

b) The amount of unbound DNA oligonucleotide in the supernatant was determined by the supernatant depletion assay. The amount of DNA bound in the S^{BL} materials was then calculated by using "*(Initial amount of DNA - unbound amount of DNA)/Volume of S^{BL} material*".

c) The amount of unbound protein-DNA conjugate was determined by the supernatant depletion assay using western blot quantification of the protein in the supernatant. The amount of protein-DNA conjugate bound in the S^{BL} materials was then calculated by using "*(Initial amount of conjugate - unbound amount of conjugate)/Volume of S^{BL} material*".

d) The amount of unbound DON was determined by the supernatant depletion assay using qPCR, so that the amount of bound DON in S^{BL} materials was then calculated by using "*(Initial amount of DON - unbound amount of DON)/Volume of S^{BL} material*".

Discussion

The total number of loops (L) is substantially greater than the number of accessible binding sites (as determined for the DNA oligonucleotide cL, and DON, protein-DNA conjugates STV-cL, VEC-cL, or DON. The fact that only less than 1% of the binding sites are accessible for hybridization can be explained by the strong shielding effect created by the dense packaging of the DNA strands during gelation, which has even been described as a kind of crystallization.[10]

Table S3. Unmodified staple strands used for the assembly of DON. The staples selected for modification of the lower or upper side of DON are indicated on the third column, as well as the modification and the position on the strand. “Lower” and “upper side” refers to the position on DON plane after binding on a surface.

Name	Sequence (5'-3')	Modification
3[256]-5[255]	GCCACCACGAAACCATCGATAGCAAAAGGGCG	Upper side (5'Cy5)
11[128]-13[127]	GCTATATTGCAAAATTAAGCAATACAGTCAAA	Upper side (5'Cy5)
19[128]-21[127]	ACTCGTCGAGGGCTTAAGCTACGTTGCGTTGC	Upper side (5'Cy5)
12[79]-10[80]	ATTATGACGAGTAGATTTAGTTTGTGAATATA	Upper side (5'Cy5)
7[320]-9[319]	AAGTAAGCTACAAAATAAACAGCCACGCTAAC	Upper side (5'Cy5)
19[192]-20[176]	GCAACAGTAGGCGGTCAGTATTAAAATCATTT	Upper side (5'Cy5)
16[47]-14[48]	CCTCAGGAATTTAAATTGTAAACGAGAAAAGC	Upper side (5'Cy5)
4[47]-2[48]	AGCCGGAACCTCAGCAGCGAAAGATTGCAGGG	Upper side (5'Cy5)
22[47]-20[48]	GTTTGCCCCAATTCCACACAACATGTCATAGC	Upper side (5'Cy5)
20[79]-18[80]	CCGAGCTCGCTCGCCCTGGAGTGATGGTTGTG	Upper side (5'Cy5)
3[192]-4[176]	TTCGGTCACTGTAGCGCGTTTTCAGAGGCAAA	Upper side (5'Cy5)
11[256]-13[255]	TAATGCAGAGTAGGGCTTAATTGAGTTAATTT	Upper side (5'Cy5)
19[320]-21[319]	GGAAGGTTGTAAGAATACGTGGCATCTGGCCA	Upper side (5'Cy5)
1[320]-3[319]	CTGAGACTAGAGCCGCCGCCAGCAGAGCCACC	Upper side (5'Cy5)
12[175]-11[191]	TTAACATCTCAATTCTACTAATAGAGAGAATA	Upper side (5'Cy5)
13[320]-15[319]	AGAATAAATTTTCCCTTAGAATCCGAATAACC	Upper side (5'Cy5)
3[128]-5[127]	CATTAAACATACCAAGCGCGAAACTCAAGAGT	Upper side (5'Cy5)
10[47]-8[48]	ACTAAAGTGAGAATGACCATAAATTTGAATCC	Upper side (5'Cy5)
4[79]-2[80]	GTCGAAATGGTAGCAACGGCTACAACGCATAA	Upper side (5'Cy5)
19[256]-21[255]	TCAAATATAGCCCTAAAACATCGCTCTGAAAT	Upper side (5'Cy5)
6[79]-4[80]	GATTTTAAGACAGATGAACGGTGTTAAATTGT	
10[175]-9[191]	AACTCCAATCAAAGCGAACCAGACTTATCCGG	
14[47]-12[48]	CCCAAAAATTAGAACCCTCATATACGGGAGAA	
1[160]-3[159]	AAAAAAAATATCAGCTTGCTTTCGTGCCACTA	
7[192]-8[176]	CAAAGTCAGGAGAATTAACTGAACTTACCAGA	
11[304]-9[303]	GAAAAATATCCTTATCATTCCAATCCTGAA	
2[271]-0[272]	TATTCACAATGCCCCCTGCCTATTTGATATAA	
12[343]-13[343]	TTTTTTAATTACTAGACACCGGAATCATTTTT	
1[96]-3[95]	TGAGAATAACAACAACCATCGCCCGAGGCTTT	
1[224]-3[223]	GGGTCAGTAAGCGCAGTCTCTGAGTTTGCC	
3[304]-1[303]	CACCCTCATTGACAGGAGGTTGATGAAACA	
8[143]-6[144]	TGCAAAAAAAGGAATTACGAGGACCAGAAC	
4[271]-2[272]	TCACCAATCGGAACCGCCTCCCTCGGCCTTGA	
8[271]-6[272]	TTTAACGTAGAGCAAGAAACAATGGGCATGAT	
23[224]-22[240]	TTGCTTTGACGAGCACGTATAACGCATCACTT	
22[63]-23[71]	AAGCGGTCGGCAAAATCCCTTA	
0[327]-1[319]	AGCGGGGTTTTGCTCATTAAGAGG	
6[343]-7[343]	TTTTTCAAAGTTACCAAGATAGCCGAATTTTT	

16[271]-14[272]	TACCTTTTAATTACCTTTTTTAATGCTGAGAA
17[224]-19[223]	CATCATATCTTTGCCCGAACGTTGCAGCAA
10[343]-11[343]	TTTTTAGAAACCAATCTACGAGCATGTTTTTT
5[256]-7[255]	ACATTCAACTTATTACGCAGTATGGTTAAGCC
13[24]-12[24]	TTTTTGATAAAAATTTTTCAACGCAAGTTTTT
23[24]-22[24]	TTTTTAATCCTGTTTGCAGCAGGCGAATTTTT
13[128]-15[127]	TCACCATCAGTCTGGAGCAAACAAAACGCCAT
9[192]-10[176]	TATTCTAAATCAGATATAGAAGGCCGGAAGCA
18[111]-16[112]	AGCCAGGGGTTGGGAAGGGCGATCGGTCACGT
21[128]-23[127]	GCTCACTGGTTTTTCTTTTCACCAGAACAAGA
10[143]-8[144]	TAATTGCAGACTTCAAATATCGGAGGCTTT
17[128]-19[127]	CCTCTTCGCCAGTGCCAAGCTTTCTGTAAGCA
10[111]-8[112]	GCGGATGGATTGCATCAAAAAGATGGGTAATA
2[239]-0[240]	AGAATGGAGCCTTGAGTAACAGTGCCGTACTC
5[24]-4[24]	TTTTTCGGTCAATCATCGAGGCGCAGATTTTT
12[63]-14[64]	ACTTTTGTTTTAAATGCAATGCCCCCGGTT
0[271]-1[255]	GTATAGCCCGGAATAGGTGTATCACCCGTATA
14[239]-12[240]	CTGAGAGATTTCAAATATATTTTAGAATCGCC
23[72]-22[80]	TAAATCAAAAGAATAGGCCTGGCC
10[239]-8[240]	TTCATCGTTTGCGGGAGGTTTTGAAGCCTTTA
7[256]-9[255]	CAATAATACAAAAATGAAAATAGCAGCCTTAA
21[192]-22[176]	GCCATTGCATATCCAGAACAATATCGGCCAAC
6[271]-4[272]	TAAGACTCCCGATTGAGGGAGGGACGGAAACG
21[320]-23[327]	ACAGAGATTCAGTGAGGCCACCGAGGAACGGTACGCCAG A
14[111]-12[112]	ATGAACGGTGAGAAAGGCCGGAGAAAGCCTCA
12[143]-10[144]	AGAATTATTCATTTGGGGCGCGAGTACCTT
15[24]-14[24]	TTTTTTATAAGCAAATCAGGAAGATTGTTTTT
4[111]-2[112]	ACGGAGATAAGACTTTTTCATGAGAGTTGCGC
17[208]-15[207]	AAGGAGCGAGTTACAAAATCGCGCAAAAGAAG
21[224]-23[223]	TACCTACAAGAAGAACTCAAACTACTATGG
20[343]-21[343]	TTTTTGACCTGAAAGCAGAACCCTTCTTTTTT
7[24]-6[24]	TTTTTCTACGTTAATAGGAAGAAAAATTTTTT
14[79]-12[80]	CATATGTACTGAGTAATGTGTAGGCCAAAAAC
4[143]-2[144]	AGCGATTGGGTAAAATACGTAAAGGTGAAT
15[192]-16[176]	ACCTGAGCAGAGGCGAATTATTCAGAGCGAGT
18[175]-17[191]	CGCCAGGGCTGCAAGGCGATTAAGTTTGCGGA
12[111]-10[112]	GAGCATAACGCAAATGGTCAATAATCATTTTT
1[288]-3[287]	TATTATTCGGCAGGTCAGACGATTAGAGCCGC
17[24]-16[24]	TTTTTCAGCCAGCTTTAGATCGCACTCTTTTT
10[79]-8[80]	ATGCTGTATACCCTGACTATTATATCCAATAC
13[96]-15[95]	CAAAAGGGTAATCGTAAAACTAGCTTGTTAAA
2[79]-0[72]	CCGATATACAACTTTCAACAGTTTAGCATTCCACAGACA G

0[111]-1[95]	ACCAGTACAAACTACAACGCCTGTCAGCGGAG
16[111]-14[112]	TGGTGTAGTTTTTTAACCAATAGGGAGAATCG
22[111]-20[112]	GGCAACAGGCTAACTCACATTAATGGTGCTTG
0[143]-1[127]	GAACCCATGTACCGTAACACTGAGGAATTGC
17[288]-19[287]	CTTCTGAAATAATACATTTGAGGAATCTGGTC
21[96]-23[95]	ATGAGTGACTGATTGCCCTTCACCCCCGAGAT
21[256]-23[255]	GGATTATTCTTTGATTAGTAATAATGCTTTCC
6[111]-4[112]	TTCAACTTGGCTGGCTGACCTTCAAAAGTACA
9[208]-7[207]	GGCGTTTTGGAAGCGCATTAGACGGAGGGTAA
18[271]-16[272]	TATTAGACATAATCCTGATTGTTTAGTAACAG
23[128]-22[144]	GTCCACTATTAAAGAACGTGGACTTGGGCGC
6[143]-4[144]	GAGTAGTCAAGAACCGGATATTTGACCCCC
8[47]-6[48]	CCCTCAAAAAACGAACTAACGGAAACCAGTCA
8[79]-6[80]	TGCGGAATTAGAAAGATTCATCAGCCTTATGC
11[24]-10[24]	TTTTTAAGTTTCATTCACGGTGTCTGGTTTTT
8[111]-6[112]	GTAAAATGCCACATTCAACTAATGGGTTTAAT
9[256]-11[255]	ATCAAGATGAGAACAAGCAAGCCGTGTTCAGC
18[63]-20[64]	TTAAGTGTTCTAATCTATTTACGAATTCGT
22[143]-20[144]	CAGGGTGCCCGCTTTCCAGTCGGTGTAATG
1[208]-0[200]	TAATAAGTCAGAACCGCCACCCTC
3[320]-5[319]	ACCCTCAGATTAGAGCCAGCAAAAGGTGAATT
10[271]-8[272]	CACTCATCTAGTTGCTATTTTGCAATTTTTTG
21[160]-23[167]	GTCGTGCCAGAGGCGGTTTGCGTATCCAACGTCAAAGGG C
15[96]-17[95]	TCAGCTCAATGGGCGCATCGTAACTTCAGGCT
20[47]-18[48]	TGTTTCCTGCCAAAATAACCCCGCTCCTTAGT
4[239]-2[240]	ATCAGTAGATAATCAAAATCACCGTTAAAGCC
11[320]-13[319]	TCCTAATTAAAAGCCTGTTTAGTAGTTAAATA
15[304]-13[303]	ATGTGAGTTTGAAAACATAGCGATGTGATA
13[224]-15[223]	GAAAACTTCTACCTTTTTAACCTACAAACA
0[343]-1[343]	TTTTTGGATTAGGATTCCTCAAGAGAATTTTT
13[160]-15[159]	CTGATAAAATCTACAAAGGCTATCTCCTGTAG
22[343]-23[343]	TTTTTTGTTTTTATAAATCCTGAGAAGTTTTT
14[63]-16[64]	GATAATCTTAATATTTTGTTAATGAGGGGA
15[224]-17[223]	TCAAGAAATTGCTTTGAATACCAGAATTAT
23[96]-22[112]	AGGGTTGAGTGTTGTTCCAGTTTGGTGAGACG
20[271]-18[272]	GAACTGATCAAACCCTCAATCAATTTTAGAAG
8[343]-9[343]	TTTTTAATTTGCCAGTTTCCAGAGCCTTTTTT
17[304]-15[303]	AGGGTTAGGATTTTCAGGTTTAATCAATAT
18[143]-16[144]	ACGACGGCTATTACGCCAGCTGGAACAAAC
23[40]-22[48]	ATGGTGGTTCCGAAATCCACGCTG
11[208]-9[207]	GGTAAAGTGCCCAATAGCAAGCAAGAACGCGA
16[143]-14[144]	GGCGGATATTCGCGTCTGGCCTAGGTCATT
18[79]-16[80]	AATTCATGAAAGCGCCATTCGCCACGTGCATC

19[24]-18[24]	TTTTTGACAATGTCCCGTCAACCTTATTTTTT
20[143]-18[144]	AGTAAACGTGGGCACGAATATAGTTGTAAA
0[71]-2[64]	CCCTCATAGTTAGCGTGGGATTTTGCTAAATTCGGTCG
16[79]-14[80]	TGCCAGTTAATTCGCATTAAATTTATGTCAAT
14[271]-12[272]	GAGTCAATGACCTAAATTTAATGGAAAGCCAA
23[256]-22[272]	TCGTTAGAATCAGAGCGGGAGCTACGTTGTAG
9[320]-11[319]	GAGCGTCTAATAATCGGCTGTCTTATATCCCA
5[192]-6[176]	GTTTATTTAAACGCAAAGACACCATTCAGTGA
2[143]-0[144]	TTCTTAAATTTTTTCACGTTGACCCAATAG
6[239]-4[240]	CGTAGAAAACCAGCGCCAAAGACAGCACCGTA
14[143]-12[144]	GCCTGAGAATATGATATTCAACCAAGGCAA
9[96]-11[95]	CAAAGCGGCTTAGAGCTTAATTGCACCATTAG
21[24]-20[24]	TTTTTTTATCCGCTCAGTGTGAAATTGTTTTT
18[47]-16[48]	GCTGAATTCCGGCACCGCTTCTGGAGTATCGG
9[304]-7[303]	TCTTACCAATATTATTTATCCCACTTACCG
15[320]-17[319]	TTGCTTCTATAAAGAAATTGCGTAAACCTACC
5[128]-7[127]	AATCTTGAAAATTGGGCTTGAGATCAGATACA
1[256]-3[255]	AACAGTTAAACAAATAAATCCTCAGAACCAGA
2[63]-4[64]	CTGAGGCCAGCATCGGAACGAGCCGCGACC
15[160]-17[159]	CCAGCTTTGTCGGATTCTCCGTGGGCGAAAGG
22[175]-21[191]	GCGCGGGGAGCTGCATTAATGAATTACCGCCA
21[288]-23[295]	ACGACCAGCATCACGCAAATTAACAACAGGAGGCCGATTA
22[79]-20[80]	CTGAGAGAAAAGTGTAAAGCCTGGCCCGGGTA
13[192]-14[176]	TGCAAATCTATATAACTATATGTATAGCTATT
5[304]-3[303]	TCATTAAATCACCAGTAGCACCAGAACCGC
21[208]-19[207]	AAACGCTCAGATAAAACAGAGGTGGCCACGCT
20[175]-19[191]	CTCCGAACTGACGCATTTCACATACACCGCCT
8[63]-10[64]	ATATTCACAAAAATCAGGTCTTGCTCAACA
16[239]-14[240]	TCGCCTGAACAAAATTAATTACATTCATAGGT
13[256]-15[255]	CATCTTCTAGTGAATTTATCAAAATTAACAAT
21[304]-19[303]	AGGGACATCAGACAATATTTTTGAATCAAC
17[96]-19[95]	GCGCAACTTGGATGTTCTTCTAAGCTCTATGA
20[63]-22[64]	AATCATGACGAGCCGGAAGCATGTTGCAGC
22[239]-20[240]	GCCTGAGTTTTTGACGCTCAATCGCATTAAAA
2[47]-0[40]	AGTTAAAGAAATGAATTTTCTGTATAACGATCTAAAGTTT
1[128]-3[127]	GAATAATAACAGCTTGATACCGATGAAGTTTC
20[111]-18[112]	TTACCTCGGTGCGGCCCTGCCATCTCAGGAGA
22[271]-20[272]	CAATACTTTACATTGGCAGATTCATAATGCGC
23[200]-21[207]	CCGCTACAGGGCGCGTATCGGCCTTGCTGGTAAACAGGAA
23[168]-23[199]	GAAAAACCGTCTATCACGCCGCGCTTAATGCG
6[47]-4[48]	GGACGTTGAAGGGAACCGAACTGATGTTACTT
15[256]-17[255]	TTCATTTGACATCGGGAGAAACAAATGGCAAT

3[24]-2[24]	TTTTTGGGATCGTCACGCCGCTTTTGCTTTTT
8[175]-7[191]	CGACGATACTATCATAACCCTCGTACCCTGAA
17[160]-19[159]	GGGATGTGTTTTCCCAGTCACGACGGGGCCTT
9[128]-11[127]	AGCCCGAATCCTTTTGATAAGAGGCCTGTTTA
5[320]-7[319]	ATCACCGTGAAGGAAACCGAGGAATTTAAGAA
7[128]-9[127]	TAACGCCAGAAGTTTTGCCAGAGGTAAGAGGA
1[24]-0[24]	TTTTTCAGACGTTAGTTGTCGTCTTTCTTTTT
14[343]-15[343]	TTTTTCTATTAATTAAGTAAATCGTCGTTTTT
15[288]-17[287]	TACATAAACGTCAGATGAATATACGGATTATA
2[175]-1[191]	TATCGGTTGGCTCCAAAAGGAGCCTTTGATGA
17[256]-19[255]	TCATCAATTTTACAAACAATTCGAGCTGAACC
12[47]-10[48]	GCCTTTATCATATAACAGTTGATTAATATGCA
1[304]-0[296]	TGAAAGTAGTACCAGGCGGATAA
3[208]-1[207]	TTATTAGCATTTACCGTTCCAGTATGTACTGG
8[239]-6[240]	CAGAGAGAAACCCACAAGAATTGATTAGCAAA
2[343]-3[343]	TTTTTGAACCACCACCAGCCGCCACCATTTTT
6[175]-5[191]	ATAAGGCTGTAACAAAGCTGCTCACGGAATAA
15[208]-13[207]	ATGATGAACCGGCTTAGGTTGGGTCAATCGCA
18[239]-16[240]	ATTAAATCTCCTGATTATCAGATGTAACGGAT
5[208]-3[207]	TCAATAGATGCCTTTAGCGTCAGATAGCCCCC
15[128]-17[127]	CAAAAATATGACCGTAATGGGATAGGTGCGGG
12[271]-10[272]	CGCTCAACAACGCGCCTGTTTATCAAGTACCG
17[192]-18[176]	ACAAAGAATGAGTAACATTATCATTTGGGTAA
0[175]-1[159]	ACCCTCATTTTCAGGGATAGCAAGAAATCTCC
4[343]-5[343]	TTTTTGCCATTTGGGACACCGACTTGATTTTT
17[320]-19[319]	ATATCAAACTAACAACTAATAGATGGAATTGA
16[343]-17[343]	TTTTTGTAAAACAGAAATTATTTGCACTTTTT
9[24]-8[24]	TTTTTGTTCAGAAAACTGCTTTAAACATTTTT
18[343]-19[343]	TTTTTCTTTAGGAGCAATCTAAAATATTTTTT
1[192]-2[176]	TACAGGAGAGCGTCATACATGGCTTTTAATTG
12[239]-10[240]	ATATTTAAACGACAATAAACAACATTTTTATT
4[175]-3[191]	AGAATACACCAACCTAAAACGAAATCGGCATT
6[63]-8[64]	TCATTATCAACATTATTACAGGCGTCATAA
14[175]-13[191]	TTTGAGAGTTAATGCCGGAGAGGGAATGCTGA
23[296]-21[303]	AAGGGATTTTAGACAGTAAAAGAGTCTGTCTAATAAA
9[224]-11[223]	CTCCCGACAGGAATCATTACCGCAATTCTG
0[295]-1[287]	GTGCCGTCGAGAGGGTTCGGAACC
0[239]-1[223]	AGGAGGTTTAGTACCGCCACCCTTTTAACG
20[239]-18[240]	ATACCGAATCTAAAGCATCACCTTCAACTCGT
2[111]-0[112]	CGACAATGGAAAGGAACAACTAAAGTTTCGTC
9[288]-11[287]	CAATTTTAGAACGGGTATTAAACCAACAATAG
0[199]-0[176]	AGAACCGCCACCCTCAGAGCCACC
9[160]-11[159]	TTCGAGCTCAGGTCAGGATTAGAGAGCTGAAA

16[175]-15[191]	AACAACCCCATCAACATTAAATGTTTTCAATT	
13[288]-15[287]	ACCGACCGTAGCTTAGATTAAGACGGAAACAG	
7[160]-9[159]	GAGCAACAAAAACCAAAATAGCGACGTTTTAA	
11[224]-13[223]	TCCAGACGCAACGCCAACATGTAAACGCGA	
3[224]-5[223]	ATCTTTTCCGACAGAATCAAGTTAAATTCA	
5[96]-7[95]	GGCGCATATAATCATTGTGAATTATTGAGATT	
19[224]-21[223]	ATGAAAAACGAACCACCAGCAGAATGGAAA	
11[96]-13[95]	ATACATTTAGCTAAATCGGTTGTATAAAGATT	
11[160]-13[159]	AGGTGGCACAATAAATCATACAGGCGTTCTAG	
11[288]-13[287]	ATAAGTCCAAATTCTTACCAGTATTTTGAAAT	
7[96]-9[95]	TAGGAATATTTAGACTGGATAGCGGTCAGAAG	
7[224]-9[223]	AGAGAGATATAACATAAAAACAGAGCGAAC	
3[160]-5[159]	CGAAGGCACTAAAACACTCATCTTCATTACCC	
5[160]-7[159]	AAATCAACTGCCCTGACGAGAAACCATAGTAA	
19[160]-21[159]	GAATCGGCTCTGACCTCCTGGTTGGGAAACCT	
5[288]-7[287]	ATTGACGGAATACCCAAAAGAACTAAATAGCA	
7[288]-9[287]	ATAGCTATATCCAAATAAGAAACGCCCAGCTA	
5[224]-7[223]	TATGGTTTATACATACATAAAGGTAATATC	
19[304]-17[303]	AGTTGAAATAGAGCCGTCAATAGTAATGGA	Lower side (3'binding)
13[208]-11[207]	AGACAAAGATTTAGGCAGAGGCATCGACAAAA	Lower side (3'binding)
16[63]-18[64]	CGACGACTGCCGGAAACCAGGCCGCACGAC	Lower side (3'binding)
13[304]-11[303]	AATAAGGCTCATATGCGTTATACTGAACAA	Lower side (3'binding)
10[63]-12[64]	TGTTTTACCCAATTCTGCGAACCCTGTAAT	Lower side (3'binding)
4[63]-6[64]	TGCTCCACCAACTTTGAAAGAGGAACTGGC	Lower side (3'binding)
19[208]-17[207]	GAGAGCCAATTAATTTTAAAAGTTACCACCAG	Lower side (3'binding)
7[304]-5[303]	AAGCCCTTACGCAATAATAACGGAAATTAT	Lower side (3'binding)
7[208]-5[207]	TTGAGCGCTGGCAACATATAAAAGTGTCACAA	Lower side (3'binding)
11[192]-12[176]	TAAAGTACTTTCGAGCCAGTAATATAGTAGCA	Upper side (5'biotin)
19[96]-21[95]	TACCGACAATAAAGACGGAGGATCGGTGCCTA	Upper side (5'biotin)
3[96]-5[95]	GAGGACTATTGTATCATCGCCTGAACAGACCA	Upper side (5'biotin)
19[288]-21[287]	AGTTGGCAAATGGCTATTAGTCTTCCAGTCAC	Upper side (5'biotin)
3[288]-5[287]	CACCCTCATTACCATTAGCAAGGCAGGTAAAT	Upper side (5'biotin)

Table S4. Protruding modified staple strands used for the assembly of DON and the binding to the loop sequence (L) in S^{BL} materials.

Name	Sequence (5'-3')
19[304]-17[303]-(CAT)8	AGTTGAAATAGAGCCGTCAATAGTAATGGATTTTTTTTCATCATCATCATCATCATCATCAT
13[208]-11[207]-(CAT)8	AGACAAAGATTTAGGCAGAGGCATCGACAAAATTTTTTTTCATCATCATCATCATCATCATCAT
16[63]-18[64]-(CAT)8	CGACGACTGCCGGAAACCAGGCCGCACGACTTTTTTTTCATCATCATCATCATCATCATCAT
13[304]-11[303]-(CAT)8	AATAAGGCTCATATGCGTTATACTGAACAATTTTTTTTCATCATCATCATCATCATCATCAT
10[63]-12[64]-(CAT)8	TGTTTTACCCAATTCTGCGAACCCTGTAATTTTTTTTTCATCATCATCATCATCATCATCAT
4[63]-6[64]-(CAT)8	TGCTCCACCAACTTTGAAAGAGGAACTGGCTTTTTTTTCATCATCATCATCATCATCATCAT
19[208]-17[207]-(CAT)8	GAGAGCCAATTAATTTTAAAAGTTACCACCAGTTTTTTTTCATCATCATCATCATCATCATCAT
7[304]-5[303]-(CAT)8	AAGCCCTTACGCAATAATAACGGAAATTATTTTTTTTTCATCATCATCATCATCATCATCAT
7[208]-5[207]-(CAT)8	TTGAGCGCTGGCAACATATAAAAGTGTCACAATTTTTTTTCATCATCATCATCATCATCATCAT

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